Lipid Changes in the Eye Concomitant with the Development of Atherosclerosis in the Aorta in the Rabbit

By Henry G. Roscoe, Ph.D., and Adolph W. Vogel, M.D.

ABSTRACT
The lipid changes that occur in the eyes and aortas of male Dutch belted rabbits maintained on 1% dietary cholesterol for 1 to 3 months were studied. In the cornea, iris, ciliary body, and aorta, the most noteworthy lipid change was in tissue cholesterol, which increased with increased time of feeding cholesterol. The phospholipid content of all the tissues increased to a lesser extent but followed the same pattern as cholesterol with respect to time. No changes in tissue triglyceride could be detected during the experimental period. The iris had the capacity to accumulate large amounts of cholesterol. The total iridic tissue (average wet weight, 75 mg/rabbit) accumulated an average of 10.2 mg of cholesterol after the rabbit had eaten a 1% cholesterol diet for 3 months; the average increase was 14.2 mg in aortic tissue (average wet weight = 380 mg/rabbit). The severity of disease graded visually correlated with the cholesterol concentration in the cornea and aorta. Using cholesterol concentration as the criterion, no significant correlation between aortic and corneal disease was found (P > 0.10), but a good degree of correlation (P < 0.01) existed between the severity of iridic and aortic involvement over the 3-month experimental period.

ADDITIONAL KEY WORDS
triglyceride cornea iris cholesterol ciliary body phospholipid

It has been known for many years that rabbits given dietary cholesterol develop eye lesions in addition to aortic atherosclerosis (1). Cogan and Kuwabara (2) and Janes (3) described the changes that occur in different parts of the rabbit eye as a result of cholesterol feeding. Histologically, the lesions in both the eye and in the aorta were characterized by the accumulation of large amounts of sudanophilic material and the presence of numerous foam cells. These findings suggested that there might be a relationship between the development of the eye and aortic lesions in cholesterol-fed rabbits. In an attempt to find out if such a relationship exists, we investigated the development of lesions in various parts of the eye and aorta with respect to their lipid composition.

Methods
Animals.—Male Dutch belted rabbits1 weighing 1260 to 1800 g were divided into two groups. Five animals were fed ground rabbit pellets2 for 3 months and served as control animals. The other group, consisting of 15 animals, was fed the same diet plus 1% cholesterol; cholesterol3 was dissolved in chloroform and then mixed with the food, and the chloroform was then allowed to evaporate. All animals were allowed food and water ad libitum during the experiment.

Corneal changes were studied with either the unaided eye or a binocular eye-loupe (Binocular Loupe4) (2x) at 2-week intervals during the 97-day experimental period. To establish a semi-quantitative measure of corneal disease, an arbitrary grading system was devised. The grading system used is outlined in Table 1. The grading was performed by two observers who had no knowledge of the prior treatment of the animals.

From the Department of Metabolic Chemotherapy, Experimental Therapeutics Research Section, Lederle Laboratories Division, American Cyanamid Company, Pearl River, New York 10965.
Accepted for publication September 24, 1968.

1Research Animal Center, Middletown, New York.
2W. F. Fisher and Son, Bound Brook, New Jersey.
3Cholesterol, U.S.P., Merck and Co., Rahway, New Jersey.
4Clay-Adams, Inc., New York, N. Y.
Plasma Samples.—Following the topical application of one drop of a 0.5% solution of tetracaine hydrochloride in saline to the eye, approximately 2 ml of venous blood was drawn from behind the eye via retrobulbar puncture (4) into heparinized tubes on days 0, 28 (1 month), 62 (2 months), and 97 (3 months). Plasma was obtained by centrifugation of heparinized blood at 3000 rpm for 10 minutes. Plasma extracts were prepared by pipetting 0.5 ml of plasma into 5 ml of absolute ethanol. The mixture was taken to boiling, allowed to cool, and filtered into a 10-ml volumetric flask. The precipitate was washed with an additional 4 ml of warm ethanol; the ethanolic extracts were pooled and the volume was adjusted to 10 ml with ethanol.

Since the use of retrobulbar puncture as a method for blood sampling involves the rupture of the ophthalmic venous plexus, the eyes were examined weekly for evidence of impaired blood flow. No chemosis or conjunctival injection, indicative of impaired orbital circulation, was seen on physical examination. Funduscopic examination revealed no evidence of retroarterial constriction or venous engorgement, indicating that the bleeding technique used did not interfere with the blood supply to the eye. In addition, gross examination of the eye just after the animal was killed failed to reveal the presence of scar tissue or residuum of old hemorrhage in the sclera.

Tissue Samples.—Four cholesterol-fed animals were killed after 1 month and four after 2 months. At the end of the third month, seven cholesterol-fed and four control animals were killed. All animals were killed by cervical fracture, and the eyes and aorta were immediately removed.

The cornea, iris, and ciliary body were carefully isolated. Visibly abnormal areas of the cornea were separated from the remainder of the tissue. Tissues were rinsed with physiological saline, blotted, weighed, and placed in 5 ml of absolute ethanol. The samples were brought to boiling and filtered. The tissues were extracted with two additional 2-ml volumes of hot ethanol, and the extracts were combined. After removing the ethanol under nitrogen at room temperature, the residues were transferred into volumetric flasks with three 3-ml volumes of hexane, and the total volume was adjusted to 10 ml with hexane.

Aortas (consisting of the arch, thoracic, and abdominal areas) were carefully stripped of all adhering tissues, slit longitudinally, and graded according to Duff and McMillan (5) using unstained tissue to avoid interference with subsequent chemical analyses. Grading was performed independently by two observers who had no prior knowledge of the treatment of the animals.

After grading, the aortas were divided into atheromatous and medial plus adventitial layers when possible. The tissues were rinsed with physiological saline, blotted, weighed, and extracted with 175 ml of absolute ethanol in a Soxhlet apparatus for 24 hours. The ethanol was evaporated under nitrogen in a steam bath, and the residue was extracted with four 10-ml volumes of n-hexane. The hexane extracts were combined and brought to a total volume of 50 ml for analysis of lipid.

Chemical Analyses.—Cholesterol was determined according to Searcy and Bergquist (6) following saponification in alcoholic KOH (7).

Total lipid phosphorus was determined by the method of Bartlett (8). Phospholipid was calculated by multiplying the lipid phosphorus by 25. Triglyceride was measured by the method of Van Handel and Zilversmit (9).

Extraction Method.—Methods for the extraction of lipids from animal tissues commonly in use require that the tissue be finally divided by homogenization, shredding in a blender, or grinding. In tissues that are relatively rich in
Comparison of Lipid Extraction Methods

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Cholesterol (mg/g)</th>
<th>Phospholipid (mg/g)</th>
<th>Cholesterol (mg/g)</th>
<th>Phospholipid (mg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Left eye*</td>
<td></td>
<td>Right eye†</td>
<td></td>
</tr>
<tr>
<td>Cornea</td>
<td>0.42</td>
<td>2.03</td>
<td>0.38</td>
<td>1.98</td>
</tr>
<tr>
<td></td>
<td>0.41</td>
<td>1.83</td>
<td>0.40</td>
<td>1.72</td>
</tr>
<tr>
<td></td>
<td>0.37</td>
<td>2.50</td>
<td>0.35</td>
<td>2.37</td>
</tr>
<tr>
<td>Iris</td>
<td>2.47</td>
<td>8.20</td>
<td>2.48</td>
<td>7.66</td>
</tr>
<tr>
<td></td>
<td>2.51</td>
<td>8.03</td>
<td>2.31</td>
<td>8.10</td>
</tr>
<tr>
<td></td>
<td>1.96</td>
<td>7.70</td>
<td>2.05</td>
<td>7.30</td>
</tr>
</tbody>
</table>

*Extracted with hot ethanol; †extracted with chloroform-methanol; ‡concentration in mg/g wet weight of tissue.

connective tissue, grinding the tissue is a troublesome and time-consuming process. During this experiment, it was found that hot alcohol could be satisfactorily used as a lipid extractant directly on intact eye tissue. The efficacy of the hot alcohol extraction method was tested by comparison with chloroform-methanol extraction. Eyes were obtained from three normal rabbits, and the cornea and iris of the left eye were extracted with hot ethanol, as described above, while the corresponding tissues of the right eye were extracted according to Folch et al (10). The cholesterol and phospholipid contents of the extracts were determined and the results are shown in Table 2.

Statistical Methods.—Data in which visual grading was employed was analyzed using Spearman's rank correlation coefficient. All other data were analyzed using the t-test. Values of P > 0.05 were not considered significant.

Results

PLASMA

The inclusion of 1% cholesterol in the diet resulted in a rapid elevation of plasma cholesterol in 14 of the 15 animals. The one rabbit that did not respond and one control rabbit that died during the experimental period were not included in this study. The average plasma cholesterol, which after 1 month was 2077 mg/100 ml (range: 1200 to 3276 mg/100 ml), reached a final value at the end of the third month of 2833 mg/100 ml (range: 2374 to 3031 mg/100 ml) versus 36 mg/100 ml (range: 30 to 51 mg/100 ml) in the control animals. Plasma phospholipid concentration was also markedly elevated in the cholesterol-fed animals. Rabbits that received 1% dietary cholesterol for 3 months had an average plasma phospholipid concentration of 656 mg/100 ml (range: 580 to 815 mg/100 ml) compared to 87 mg/100 ml (range: 59 to 136 mg/100 ml) for the controls.

CORNEA

Visible Changes.—Corneal involvement was visible in all animals fed 1% cholesterol (Fig. 1). In the early stages, one or more small white areas, approximately 0.1 mm wide and 1 to 3 mm long, developed in the cornea adjacent to the limbus (junction of the cornea and sclera). The whitish areas increased in width and length and in many rabbits eventually formed a complete circle. The white band was not uniform in width, and in most cases there were discrete areas of more severe involvement in which the affected portion of the cornea was from 1 to 3 mm wide. In the more involved areas, there was also extensive vascularization. After the initial appearance of corneal involvement, which occurred between 14 and 28 days in the cholesterol-fed rabbits, there seemed to be little change in the severity of the corneal lesions in most of the animals during the second month. Although the average visual grade increased slightly at the end of 2 months, it was not significantly different (P > 0.05) from that found after 1 month. During the third month, however, the corneal lesions again became more severe, (P < 0.001, after 1 month versus after 3 months). No changes were seen in rabbits maintained on the control diet during the experimental period.

Lipid Content.—Because of the appearance of clearly visible corneal changes in the
Assessment of corneal lesions in cholesterol-fed rabbits. Abscissa: time on 1% dietary cholesterol. Ordinate: average visual grade per pair of corneas, graded according to the criteria presented in Table 1. Vertical lines indicate ± SEM.

cholesterol-fed animals, it was possible to separate the abnormal portion of the cornea from that which appeared grossly normal. Both the abnormal and normal areas of the cornea were analyzed separately for cholesterol and phospholipid. The results are presented in Table 3. The cholesterol concentration was low in the normal cornea (0.29 mg/g). After 1 month on the cholesterol-supplemented diet, there was a ninefold increase in the cholesterol concentration of the whole cornea. However, during the second month, no change was observed in either total cholesterol or cholesterol concentration of the whole cornea. The third month of cholesterol feeding was characterized by an increase in both total cholesterol content and cholesterol concentration (28-fold and 21-fold increase over control animals, respectively).

At all the time intervals studied, the increase in the cholesterol content of the whole cornea was a result of the increased cholesterol content of the abnormal area. In eyes obtained from rabbits maintained on dietary cholesterol for 3 months, the area of the cornea which appeared grossly normal, had essentially the same cholesterol content as corneas taken from the control animals.

Findings similar to those described above for cholesterol were made for phospholipid concentration (Table 3). In the abnormal portion of the cornea, there was a twofold increase in the phospholipid concentration over that found in corneas taken from control animals after 1 month of feeding. At the end of the second month, the phospholipid concentration and total phospholipid content of the abnormal area of the cornea was essentially the same as that found at the end of 1 month. After 3 months of feeding there was an increase in total phospholipid ($P < 0.001$), whereas phospholipid concentration, although elevated, was not statistically different from that at the second month. In the visually normal portions of the corneas taken from cholesterol-fed rabbits, although there seemed to be a small but constant increase in the phospholipid concentration with time, this change was not statistically significant. Unlike cholesterol, it was not possible to follow the phospholipid changes in the abnormal area by analyzing the whole cornea. Only after 2 and 3 months of feeding could significant changes in the phospholipid concentration and total phospholipid of the whole cornea, respectively, be seen.

**IRIS AND CILIARY BODY**

The Dutch belted strain of rabbit is unsuitable for direct visual inspection of the iris because the iris is pigmented. Therefore, the changes in lipid were the only means possible of assessing iridic involvement. The ciliary body was studied for comparison with the cornea and iris.

The changes in cholesterol and phospholipid contents in the iris and ciliary body as a result of cholesterol feeding are shown in
### TABLE 3

**Effect of Dietary Cholesterol on Corneal Cholesterol and Phospholipid Content**

<table>
<thead>
<tr>
<th>Diet</th>
<th>No. rabbits</th>
<th>Time (mo.)</th>
<th>Abnormal part</th>
<th>Normal part</th>
<th>Whole</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Cholesterol</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Total (mg*)</td>
<td>Conc. (mg/g)</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>4</td>
<td>3</td>
<td>0.21 ± 0.09</td>
<td>7.15 ± 3.64</td>
<td>0.09 ± 0.02</td>
</tr>
<tr>
<td>1% Chol.</td>
<td>4</td>
<td>1</td>
<td>0.22 ± 0.07</td>
<td>8.63 ± 3.77</td>
<td>0.05 ± 0.01</td>
</tr>
<tr>
<td>1% Chol.</td>
<td>6</td>
<td>3</td>
<td>1.11 ± 0.37</td>
<td>12.10 ± 1.21</td>
<td>0.03 ± 0.01</td>
</tr>
</tbody>
</table>

|      |             |            | Phospholipid   |             |       |
|      |             |            | Total (mg)     | Conc. (mg/g) |       |
| Control | 4 | 3 | 0.12 ± 0.02 | 4.06 ± 1.21 | 0.19 ± 0.02 | 2.14 ± 0.17 | 0.31 ± 0.02 | 2.16 ± 0.08 |
| 1% Chol. | 4 | 1 | 0.12 ± 0.06 | 4.22 ± 0.64 | 0.18 ± 0.01 | 2.40 ± 0.16 | 0.30 ± 0.02 | 2.94 ± 0.16 |
| 1% Chol. | 6 | 3 | 0.43 ± 0.16 | 5.24 ± 0.62 | 0.22 ± 0.04 | 2.60 ± 0.40 | 0.65 ± 0.11 | 3.05 ± 0.40 |

*Total in mg per pair of corneas (mean ± se); †mg/g wet weight tissue (mean ± se).
Significance between cholesterol-fed and control group: *P < 0.02, †P < 0.01, **P < 0.001.

### TABLE 4

**Effect of Dietary Cholesterol on the Cholesterol and Phospholipid Content of the Iris and Ciliary Body**

<table>
<thead>
<tr>
<th>Diet</th>
<th>No. rabbits</th>
<th>Time (mo.)</th>
<th>Iris Cholesterol</th>
<th>Ciliary body Cholesterol</th>
<th>Iris Phospholipid</th>
<th>Ciliary body Phospholipid</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Total (mg*)</td>
<td>Conc. (mg/g)</td>
<td>Total (mg)</td>
<td>Conc. (mg/g)</td>
</tr>
<tr>
<td>Control</td>
<td>4</td>
<td>3</td>
<td>0.12 ± 0.01</td>
<td>1.70 ± 0.12</td>
<td>2.90 ± 0.33</td>
<td>0.56 ± 0.06</td>
</tr>
<tr>
<td>1% Chol.</td>
<td>4</td>
<td>1</td>
<td>1.21 ± 0.24</td>
<td>16.69 ± 2.16</td>
<td>21.05 ± 2.57</td>
<td>0.75 ± 0.03</td>
</tr>
<tr>
<td>1% Chol.</td>
<td>4</td>
<td>2</td>
<td>2.30 ± 0.44</td>
<td>29.00 ± 4.06</td>
<td>27.01 ± 2.18</td>
<td>0.87 ± 0.10</td>
</tr>
<tr>
<td>1% Chol.</td>
<td>6</td>
<td>3</td>
<td>10.26 ± 1.60</td>
<td>67.10 ± 4.97</td>
<td>57.00 ± 3.56</td>
<td>2.69 ± 0.43</td>
</tr>
</tbody>
</table>

*Total in mg per pair of irises (mean ± se); †mg/g wet weight tissue (mean ± se).
Significance between cholesterol-fed and control group: *P < 0.01, †P < 0.001, ‡P < 0.02.
Visual assessment of atherosclerosis in aortas from rabbits fed 1% dietary cholesterol. The atheromas were graded by the method of Duff and McMillan (5) using unstained aortas.

Table 4. Values for total cholesterol or phospholipid of the ciliary body are not reported due to the difficulty of removing this tissue completely.

During the first 2 months there was a linear increase in the total iridic cholesterol which averaged 1.09 mg per pair of irises per month in cholesterol-fed animals. The biggest increase in total cholesterol, observed during the third month, was 7.96 mg cholesterol per pair of irises. The increase in total cholesterol was also reflected in the cholesterol concentration. The concentration of cholesterol in the ciliary body was essentially the same as that in the iris for all time periods studied. Changes in iridic content of phospholipid were much less pronounced than those of cholesterol. In spite of this, the total phospholipid and phospholipid concentration was increased significantly by the end of the first month. However, the biggest increase in iridic phospholipid was found during the third month of cholesterol feeding (1.57 mg phospholipid per pair of irises). As for cholesterol, the phospholipid concentrations in the iris and ciliary body were essentially the same during the course of the experiment.

AORTA

Visual Grading.—The degree of atherosclerosis was assessed separately in the arch, thoracic, and abdominal aorta after 1, 2, and 3 months of cholesterol feeding. As shown in Figure 2, the degree of involvement present after 1 month was minimal. By the end of the second month, the aortas were extensively involved and animals that received cholesterol for 3 months were the most severely affected. At all time periods examined, the arch was the most involved, the thoracic aorta was the least affected, and the abdominal aorta was intermediate in severity between the arch and thoracic aortas. No visible disease was seen in aortas from control animals.

Aortic Lipids.—When possible (in animals fed cholesterol for 2 or 3 months) the atheromas were separated from the remainder of the aorta (media plus adventitia), and the two tissues were analyzed independently. The results are presented in Table 5. No significant difference was found in either the cholesterol or phospholipid content of the aorta after 1 month of cholesterol feeding. After the second and third months, there was a 5-fold and 13-fold increase, respectively, in the total cholesterol content of the whole aorta over that of the control animals. The major portion of the total cholesterol was found in the atheroma, whereas the total phospholipid was equally divided between the atheromatous tissue and the media plus adventitial layer. The concentration of both cholesterol and phospholipid was greatly elevated in the atheroma compared to the media plus adventitia.

Comparison of visual grade with cholesterol concentration in cornea

It has been shown by other workers that the visual assessment of aortic lesions is correlated with the cholesterol content of the aorta expressed either as total cholesterol (mg

Circulation Research, Vol. XXIII, November 1968
### TABLE 5

**Effect of Dietary Cholesterol on Aortic Cholesterol and Phospholipid Content**

<table>
<thead>
<tr>
<th>Diet</th>
<th>No. rabbits</th>
<th>Time (months)</th>
<th>Atheroma</th>
<th>Media + Adventitia</th>
<th>Whole aorta</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Total (mg*)</td>
<td>Conc. (mg/g)</td>
<td>Total (mg)</td>
</tr>
<tr>
<td>Control</td>
<td>4</td>
<td>3</td>
<td>1.25 ± 0.37</td>
<td>3.2 ± 0.78</td>
<td></td>
</tr>
<tr>
<td>18 Chol.</td>
<td>4</td>
<td>1</td>
<td>1.66 ± 0.20</td>
<td>4.5 ± 0.53†</td>
<td>6.30 ± 1.47§</td>
</tr>
<tr>
<td>18 Chol.</td>
<td>4</td>
<td>2</td>
<td>3.80 ± 0.48</td>
<td>9.80 ± 0.93</td>
<td>15.30 ± 1.63¶</td>
</tr>
<tr>
<td>18 Chol.</td>
<td>6</td>
<td>3</td>
<td>4.40 ± 1.41</td>
<td>8.04 ± 9.90</td>
<td>8.30 ± 1.47§</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th>Phospholipid</th>
<th>Media + Adventitia</th>
<th>Whole aorta</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Atheroma</td>
<td>Media + Adventitia</td>
<td>Whole aorta</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Total (mg)</td>
<td>Conc. (mg/g)</td>
<td>Total (mg)</td>
</tr>
<tr>
<td>Control</td>
<td>4</td>
<td>3</td>
<td>1.18 ± 0.22</td>
<td>3.09 ± 0.35</td>
<td></td>
</tr>
<tr>
<td>18 Chol.</td>
<td>4</td>
<td>1</td>
<td>1.10 ± 0.14</td>
<td>2.60 ± 0.23</td>
<td></td>
</tr>
<tr>
<td>18 Chol.</td>
<td>4</td>
<td>2</td>
<td>0.77 ± 0.66</td>
<td>5.20 ± 1.24</td>
<td>1.50 ± 0.18§</td>
</tr>
<tr>
<td>18 Chol.</td>
<td>6</td>
<td>3</td>
<td>2.40 ± 0.45</td>
<td>18.93 ± 1.44</td>
<td>4.43 ± 0.61**</td>
</tr>
</tbody>
</table>

*Mean ± se; †mg/g wet weight tissue (mean ± se).
Significance between cholesterol-fed and control group: †P < 0.03, ‡P < 0.02, §P < 0.001, **P < 0.01.
Comparison of visual grading and cholesterol concentration of cholesterol-induced disease in the cornea, A, and aorta, B. Abscissa: cholesterol concentration in mg/g wet weight of tissue. Ordinate: A, average visual grade per pair of corneas and B, visual grade per aorta, average of arch, thoracic, and abdominal segments. The symbols used represent animals on 1% dietary cholesterol for 1 month (solid circles), 2 months (open circles), and 3 months (solid triangles). The above data were analyzed using Spearman's rank correlation coefficient: in A, $r_s = 0.9000$ and $P < 0.001$; in B, $r_s = 0.9088$ and $P < 0.001$.

Comparison of eye disease and aortic involvement in cholesterol-fed rabbits. A, aorta vs. cornea; B, aorta vs. iris. Abscissa: A, corneal cholesterol in mg/g wet weight per pair of corneas and B, iridic cholesterol in mg/g wet weight per pair of irises. Ordinate: aortic cholesterol in mg/g wet weight of whole aorta. The symbols used represent animals on 1% dietary cholesterol for 1 month (solid circles), 2 months (open circles), and 3 months (solid triangles).
/aorta) (11) or as cholesterol concentration (mg/100 g wet wt.) (12). When the visual corneal grade is plotted against the corneal cholesterol concentration in mg/g wet weight, a high degree of correlation was found at all time periods studied (Fig. 3, A). The data for the aorta are shown for comparison (Fig. 3, B).

**COMPARISON OF EYE INVOLVEMENT AND AORTIC DISEASE**

The results reported above suggested that the extent of both the eye and aortic involvement is related to the cholesterol content of these tissues. Using cholesterol concentration as a measure of atherosclerotic involvement, no significant correlation was found between corneal and aortic disease ($P > 0.1$, Fig. 4, A). However, when the cholesterol concentration of the iris versus the aorta was plotted (Fig. 4, B), the correlation between the two tissues was found to be significant ($P < 0.01$).

**Discussion**

The cholesterol-supplemented diet used in this experiment was effective in producing aortic atherosclerosis and eye disease. At all time intervals studied, namely, 1, 2, and 3 months, 100% of the animals had visible aortic disease and corneal disease. An interesting observation could be made on the development of the corneal lesion, namely, that the severity did not increase consistently with time. After 1 month on the 1% cholesterol diet, all animals had visible lesions, but during the second month, only a few corneal lesions increased in severity, whereas most seemed to remain the same. By the end of the third month, the lesions again increased in severity. It was further observed that a lesion could increase in severity from +1 to +2 within less than 2 weeks.

The distribution of aortic plaques found in this experiment differed from that normally seen in cholesterol-fed rabbits, in which the arch is the most severely involved followed by the thoracic aorta and the abdominal aortic segment, which is the least affected (13). The animals used in this study were also the most severely affected in the arch, but the abdominal aorta was consistently more involved than the thoracic aorta. In spite of this apparent anomaly, the lipid composition as well as the correlation of visual grade with cholesterol content of the aortic plaques was in agreement with the findings of others (11, 12, 14).

One way of gaining insight into the possible relationship between the visible aortic and eye disease was to determine the lipid composition of both tissues. Janes (3) concluded that the diseased eye tissue found in rabbits fed cholesterol contained large amounts of lipid on the basis of its staining properties. However, the histologic staining procedures used did not permit a determination of the nature of the lipid present. Cogan and Kuwabara (2) described the appearance of birefringent crystals in addition to sudanophilic material in the iris and ciliary body of cholesterol-fed rabbits, suggesting the presence of large amounts of cholesterol in these tissues. In the present study it was found that cholesterol is the principal lipid which accumulates in the cornea, iris, and ciliary body of hypercholesterolemic rabbits. Phospholipid also increased in these tissues, but to a much lesser degree. In the cornea, where it was possible to separate the visually diseased from the normal tissue, the increased cholesterol and phospholipid content of this tissue was localized in the diseased area only. Although corneal cholesterol, which in the control rabbits is low (0.29 mg/g) increased 20-fold (6.1 mg/g) after 3 months on the 1% cholesterol diet, the most striking increase in tissue cholesterol was found in the iris and ciliary body and reached final values of 67.1 mg/g and 57.0 mg/g, respectively. These findings are in agreement with those of Rubenstein et al. (15), who found large increases in iridic and ciliary body cholesterol in New Zealand white rabbits after 10 weeks of a 2% cholesterol diet. In the present study, the capacity of the iris to accumulate cholesterol was particularly noteworthy. Thus, the total iridic tissue, which weighed approximately 75 mg wet weight/rabbit in normal animals, accumulated an average of 10.2 mg
of cholesterol after 3 months on the cholesterol-supplemented diet compared to an average increase during the same time period of 14.2 mg in aortic tissue, which had an average wet weight of 380 mg/rabbit. It was also observed that the initial rate of cholesterol increase in all the eye parts studied exceeded that found in the aorta. Thus, the cholesterol concentration of the cornea, iris, and ciliary body increased 8.9-, 9.8-, and 7.2-fold, respectively, during the first month of cholesterol feeding compared to a 1.4-fold increase in aortic cholesterol concentration.

In general, the change in tissue phospholipid was found to parallel that of cholesterol in both the eye and aorta at all time intervals studied. In the cornea and aorta, the phospholipid changes are not apparent in the whole tissues, particularly during the first month, but when the diseased areas of these tissues were examined, the increase in phospholipid was readily seen. In contrast, the change in the phospholipid content of the iris and ciliary body could be seen even after 1 month in cholesterol-fed animals.

Attempts to determine the triglyceride content of the eye parts were unsuccessful. Even after 3 months of cholesterol feeding, no significant amount of triglyceride (less than 1 mg/g) could be found. The lack of triglyceride accumulation in eye tissues from cholesterol-fed animals is similar to that found in the diseased aorta (14). When aortas from rabbits fed 1% dietary cholesterol for 1 and 2 months were compared, there was no difference in triglyceride concentration (17.8 mg/g and 16.9 mg/g, respectively), even though the tissue cholesterol increased from 4.5 mg/g to 23.6 mg/g during this time. In addition, the triglyceride concentration of the atheromatous tissue was approximately one third that of the medial plus adventitial layer in aortas from rabbits fed cholesterol for 2 months.

The results of the present study reveal a similarity to the lipid changes in the cornea, iris, and ciliary body as compared to the aorta. Of particular interest is the increase in tissue phospholipid noted in all of the eye tissues studied. Similar findings with regard to the aortic phospholipid in cholesterol-fed rabbits have been published by Newman and Zilversmit (14) and have been interpreted by them to be a response to cholesterol infiltration, as distinct from simply a reflection of cellular proliferation.

The present study was initiated to ascertain whether the degree of lipid infiltration observed in the corneas of cholesterol-fed rabbits could be used as a reliable index of aortic atherosclerosis. The data, however, showed no correlation between aortic and corneal disease (P > 0.10), while a good degree of correlation (P < 0.01) existed between the severity of iridic and aortic involvement during the 3-month experimental period. These results support the observation of Friedman and Byers (16) of a relationship between iridic lipid infiltration and aortic atherosclerosis in cholesterol-fed albino rabbits and extend their findings to include a similarity in the lipid changes, determined by chemical analyses, which occur between the two tissues as a result of cholesterol feeding.

Acknowledgments

The authors wish to thank Dr. M. J. Fahrenbach and Mr. B. A. Riccardi for grading aortas. The technical assistance of Mr. R. Goldstein is also gratefully acknowledged.

References


Circulation Research, Vol. XXIII, November 1968
Lipid Changes in the Eye Concomitant with the Development of Atherosclerosis in the Aorta in the Rabbit

HENRY G. ROSCOE and ADOLPH W. VOGEL

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation Research can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at: http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation Research is online at: http://circres.ahajournals.org//subscriptions/
Circ Res. 1968;23:633-643
doi: 10.1161/01.RES.23.5.633
Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1968 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/23/5/633

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation Research can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation Research is online at:
http://circres.ahajournals.org//subscriptions/